mitochondria. Unlike Bensley and others, though, he continued to maintain for years that secretory particles were a major component as well:

> Secretory particles are abundant in the guinea pig liver, especially in the fasting animal, where they accumulate and seem to fill the cell completely, and it appears probable that up to the present, mitochondria have not been isolated in a pure or concentrated form, a large part of the so-called "mitochondria" fraction representing probably, to a large extent, mature secretory granules. (Claude, 1943b, p. 455)

In 1943 Claude also began to employ a different procedure, centrifuging whole liver cells from *Amphiuma tridactylum* for one hour at 18,000 *g*, causing the cell contents to separate into distinguishable layers while remaining within the cell membrane. By staining the cells with Bensley's stain (acid fuchsin-methyl green), he rendered what he took to be secretory granules and mitochondria vivid red against a purple background (Claude, 1943a). This yielded a sharp distinction between four successive layers, which Claude interpreted as (1) glycogen, (2) a combination of secretory granules and mitochondria that appeared red and also contained nuclei, (3) the "purple substance" that he took to be microsomes, and (4) the true hyloplasm (cytosol).

In general, the Bensley group welcomed Claude's entry into the field of centrifugation studies that they had pioneered. He was an invited speaker at the symposium in November 1942 in honor of Bensley's seventy-fifth birthday, and many of Bensley's colleagues referred positively to his contributions (Hoerr called his work "superb"). However, they challenged his refusal to identify his large-granule fraction as essentially mitochondrial and his insistence that many of the granules were secretory. Hoerr said, "it is obvious that he has separated the liver mitochondria" (1943), appealing to the ability of staining to differentiate between mitochondria and secretory granules when they are present in, for example, pancreas cells. He further criticized Claude's methods of separation, arguing that they failed to produce pure preparations but rather yielded mixtures. Just as Claude continued to refer to *large granules* in preference to *mitochondria*, so Bensley's group rejected Claude's term *microsomes*, instead referring to the small particles as *submicroscopic particulates*.

The following year Claude (1944) offered yet another reason for questioning whether the large granules extracted from guinea pig liver included mitochondria – they failed to stain with Janus green. Now he concluded that liver may be a poor place to look for mitochondria, and reported results of a study on leukemic cells from rats, in which he contended that the large granule fraction consisted of true mitochondria. He then generated a chemical